DATA EVALUATION RECORD
WHOLE SEDIMENT ACUTE TOXICITY INVERTEBRATES, FRESHWATER
OPPTS Guideline 850.1735

1. **CHEMICAL:** Bifenthrin PC Code No.: 128825

2. **TEST MATERIAL:** Bifenthrin technical Purity: 93.6%

3. **CITATION:**

Authors:	Picard, C.R.
Title:	10-Day Toxicity Test Exposing Freshwater Amphipods (*Hyalella azteca*) to Bifenthrin Applied to Sediment Under Static-Renewal Conditions.
Study Completion Date:	March 28, 2014
Laboratory:	Smithers Viscient 790 Main Street Wareham, MA 02571-1037
Sponsor:	Pyrethroid Working Group FMC Corporation 701 Princeton South Corporate Center Ewing, NJ 08628
Laboratory Report ID:	13656.6156
MRID No.:	49368101
DP Barcode:	420540

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, CSS-Dynamac

Signature: Christie E. Padova **Date:** 12/10/14

APPROVED BY: Teri S. Myers, Senior Scientist, CDM Smith

Signature: Teri S Myers **Date:** 02/25/15

5. **APPROVED BY:**

Signature: **Date:**

6. **STUDY PARAMETERS:**

Age of Test Organism:	8 days old
Definitive Test Duration:	10 days
Study Method:	Intermittent flow-through
Type of Concentrations:	Mean-measured sediment, bulk and OC-normalized

7. CONCLUSIONS:

Results Synopsis:

Based upon mean-measured sediment concentrations:

Survival:
LC_{50}: 41.2 µg/kg 95% C.I.: 37.2 to 44.8 µg/kg
Slope: 9.19 (6.69 to 11.7)
NOAEC: 15 µg/kg
LOAEC: 31 µg/kg

Growth (length):
EC_{50}: 94.9 µg/kg 95% C.I.: 75.2 to 120 µg/kg
Slope: N/A
NOAEC: 7.0 µg/kg
LOAEC: 15 µg/kg

Based upon OC-normalized mean-measured sediment concentrations:

Survival:
LC_{50}: 1.3 µg/g OC 95% C.I.: 1.2 to 1.4 µg/g OC
Slope: 9.19 (6.69 to 11.7)
NOAEC: 0.48 µg/g OC
LOAEC: 1.0 µg/g OC

Growth (length):
EC_{50}: 3.06 µg/g OC 95% C.I.: 2.43 to 3.87 µg/g OC
Slope: N/A
NOAEC: 0.23 µg/g OC
LOAEC: 0.48 µg/g OC

8. ADEQUACY OF THE STUDY:

A. Classification: This study [is/is not scientifically sound] and is classified as [acceptable/supplemental (quantitative)/supplemental (qualitative)/invalid].

B. Rationale:

C. Repairability:

9. MAJOR GUIDELINE DEVIATIONS:

There were no deviations from OPPTS 850.1735 guidance that would affect the scientific soundness or acceptability of this study.

10. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *H. azteca* or *Chironomus tentans*	*Hyalella azteca*
Life Stage: For *C. tentans*: third instar (9-11 days old). The instar stage of midges must be confirmed by head capsule width (approx. 0.38 mm). For *H. azteca*: 7- to 14-day old amphipods must be produced. If growth is also an endpoint, a narrower range, such as 1- to 2-day old amphipods should be used.	*H. azteca*: 8 days old; mean length of 1.34 mm (n=20 at t_0)
Supplier Brood stock can be obtained from laboratory, commercial, or government sources. (Sources obtained from the wild should be avoided unless cultured through several generations in the laboratory.)	Amphipods originated from laboratory cultures that were maintained in 20-L glass aquaria containing 15 L culture water under flow-through conditions. Both the culture water and overlying water used during the test originated from the same source.
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period: The required culture and testing temperature is 23°C. The test organisms should be cultured in the same water to be used for testing.	Adults were removed from the main culture tanks 9 days prior to test initiation and placed in *ca.* 8 L of water. Juvenile amphipods (≤24 hours old) produced by the isolated adults were then transferred to *ca.* 0.80 L of laboratory dilution water and reared for 8 days under static conditions at 24 to 25°C and 6.9 to 7.8 mg/L dissolved oxygen with gentle oil-free aeration.
Feeding:	During holding and acclimation, amphipods were fed once daily with a combination of yeast, cereal leaves, and flaked fish food suspension (YCT). At the start of acclimation, a small amount of *Ankistrodesmus falcatus*, a unicellular green algae, and flaked fish food suspension were fed to the isolated amphipods.
Pretest Mortality: A group of organisms should not be used if they appear unhealthy, discolored (eg <20% mortality 48 h before the beginning of a test).	No mortality during the 48 hours prior to test initiation.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water (overlying water) and sediment: Soft reconstituted water or water from a natural source. Tap water is acceptable if it is dechlorinated, deionized, and carbon filtered, but its use is not encouraged. Uncontaminated natural sediment is recommended.	Laboratory well water characterized as having a total hardness and total alkalinity as $CaCO_3$ of 40 to 48 mg/L and 20 to 22 mg/L, respectively, a pH range of 7.3 to 7.7, and a conductivity range of 230 to 350 µS/cm. The TOC of the dilution water source was 0.31 and 0.35 mg/L for August and September 2013, respectively. Natural freshwater sediment (Batch No. - 091312) was collected from Glen Charlie Pond, Wareham, MA and 2.0-mm wet-sieved to remove large particles and indigenous organisms.
Does water support test animals without observable signs of stress?	Yes
Quality Of Water If problems are observed in culturing or testing of organisms, it is desirable to test water quality. Particulate, TOC, COD should be <5 mg/L and residual chlorine <11 µg/L	There were no apparent problems with water quality.
Water Temperature 23°C for both species. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1°C and 3°C, respectively.	Daily: 22 to 24°C Continuous: 23 to 24°C
pH Should not vary more than 50%. Survival is best at pH >6.5 for *C. tentans.*.	5.9 to 7.0
Dissolved Oxygen Maintained between 40 and 100%.	5.1 to 7.4 mg/L (>40% saturation)

Guideline Criteria	Reported Information
Total Hardness Should not vary more than 50%. *H. azteca* are sensitive to hardness (e.g., they are not found in waters with calcium at <7 mg/L and DO at <2 mg/L).	32 to 56 mg/L as $CaCO_3$
Conductivity Should not vary more than 50%.	210 to 340 µS/cm
Sediment Characterization All sediment must be characterized for: pH, ammonia concentration of pore water, organic carbon content (total organic carbon (TOC)), particle size distribution, and percent water content.	Particle distribution – 89% sand, 8% silt, 3% clay (sand; reviewer-derived from USDA soil texture triangle) TOC – 3.1% Percent solids – 26.87% pH – 5.5 Ammonia concentration of pore water – 6.2 mg/L (as N)
Additional Sediment Analysis BOD, COD, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis.	Not reported

Guideline Criteria	Reported Information
Laboratory Spiked Sediment Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material's identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures.	Bifenthrin technical Synonyms: FMC 54800 IUPAC name: 2-methylbiphenyl-3-ylmethyl (1*RS*,3*RS*)-3-[(*Z*)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropanecarboxylate CAS name: (2-methyl[1,1′-biphenyl]-3-yl)methyl (1*R*,3*R*)-*rel*-3-[(1*Z*)-2-chloro-3,3,3-trifluoro-1-propen-1-yl]-2,2-dimethylcyclopropanecarboxylate Description: Liquid Lot no.: PL09-0251 CAS No.: 82657-04-3 Purity: 93.6% Storage: room temperature in the dark Aqueous solubility: not reported
Stock Solutions Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required.	A 10-µg/mL primary stock solution was prepared by bringing 0.011 g of test substance (0.0010 g ai) to 100 mL with acetone. From this, six individual dosing solutions were prepared (at 0.440, 0.880, 1.76, 3.52 and 7.04 µg/mL) by diluting the appropriate amount of stock solution into 25 mL acetone. The primary stock solution and all dosing solutions were clear and colorless, with no visible un-dissolved test substance. Negative and solvent controls were included in the test.

Guideline Criteria	Reported Information
Test Concentrations For Spiked Sediment For LC50 calculation, test concentrations should bracket the predicted LC50; sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer.	A jar-rolling technique was used to apply the test substance to the sediment. A 10-mL volume of the appropriate prepared dosing stock solution (in acetone) was applied to 0.050 kg of fine silica and the solvent was allowed to evaporate off for 30 minutes. The dry sand was then added to 2.0 kg of wet sediment (total of 0.5874 kg dw) in individual glass jars. Each jar was then rolled for 4 hours at *ca*. 15 rpm. The jars were stored upright at 2 to 8°C for a 14-day equilibration period. Once a week during the equilibration period and prior to being added into the replicate exposure vessels, the jars were mixed on the rolling mill for 2 hours to ensure the sediment was homogeneous. The range of nominal concentrations (7.5 to 120 µg/kg dw) used in the definitive study was selected in consultation with the Sponsor, based on historical data and previous testing conducted at Smithers Viscient.
Test Aquaria 1. Material: Glass or stainless steel or perfluorocarbon plastics. 2. Size: 300 ml high-form lipless beakers containing 100 ml of sediment and 175 ml of overlying water.	1. Glass and 40-mesh Nitex screen (for drainage) 2. 300 mL vessels containing 100 mL (*ca*. 4.0-cm layer) of sediment (equivalent to 34 g dw) and 175 mL of overlying water. The total overlying water plus sediment volume was maintained at *ca.* 275 mL.
Type of Dilution System Daily renewal or a flow-through system may be used.	Flow-through
Flow Rate 2 volume changes/day	2 volume additions/day

Guideline Criteria	Reported Information
Aeration Dilution water should be vigorously aerated prior to use so that dissolved oxygen in the overlying water remains above 40% saturation.	None reported
Photoperiod 16 hours light, 8 hours dark at 500 to 1000 lux.	16 hours light, 8 hours dark; 500 to 780 lux
Solvents Use of a solvent should be avoided since they may influence the concentration in pore water. If used,it should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used.	Acetone, 10 mL per 0.5874 kg dw sediment The acetone was allowed to completely evaporate during the mixing procedure.

D. Test Design

Guideline Criteria	Reported Information
Sediment Into Test Chambers One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be thoroughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment.	One day prior to the addition of amphipods (day -1), the test systems were established. Overlying water was gently added using a turbulence reducer, and each vessel was placed under the renewal system.

Guideline Criteria	Reported Information
Renewal of Overlying Water: Renewal of the overlying water should be conducted on day -1 prior to the addition of organisms or food on day 0. For flow-through systems, the flow rates should not vary by more than 10% between any two chambers at any time. Proper operation should be verified by calibration prior to test initiation.	The overlying water was renewed via an intermittent delivery system in combination with a calibrated water-distribution system. The test system was calibrated before and after the test, and visually inspected at least twice daily for proper functioning.
Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.	Amphipods were impartially assigned one or two at a time into intermediate test beakers until all beakers contained ten amphipods. The test was initiated when each intermediate beaker of amphipods was added to each respective test vessel.
Range Finding Test A definitive test will not be required if no toxicity is observed at concentrations of 100 mg/kg dry weight of sediment**.**	None reported.
Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.	Test vessels were observed daily for mortality and abnormal behavior.
Nominal Concentrations of Definitive Test Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. Concentrations above aqueous solubility may be used.	0 (negative and solvent controls), 7.5, 15, 30, 60 and 120 µg/kg dw

Guideline Criteria	Reported Information
Number of Test Organisms 10 organisms per test chamber are recommended. 8 replicates per treatment should be used.	80 amphipods per level, with 10 amphipods per replicate vessel and 8 biological replicates per level An additional 4 replicates per level were maintained for chemical analysis and pore water quality and analysis.
Test organisms randomly or impartially assigned to test vessels?	Yes
Feeding *C. tentans* in each test chamber are fed 1.5 ml of a 4 g/L Tetrafin7 suspension daily. *H. azteca* may be fed with a mixture of yeast, Cerophyl, and trout chow (YCT) at a rate of 1.5 mL daily per test chamber. A drop in DO levels below 2.5 mg/L may indicate over-feeding and feeding should be suspended in all treatments until DO levels increase.	Amphipods were fed a combination of yeast, cereal leaves, and flaked fish food suspension (YCT) once daily at a rate of 1.0 mL/vessel.

Guideline Criteria	Reported Information
Water Parameter Measurements Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of the test. DO should be measured daily. Temperature should be measured daily in one test chamber from each treatment. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1 and 3°C, respectively.	Total hardness, alkalinity, conductivity and ammonia were measured in each treatment level and control solution from a composite sample at Days 0 and 10. Dissolved oxygen (DO), temperature, and pH were measured in each replicate vessel on Days 0 and 10, and in one alternating replicate from each level on Days 1 to 9. In addition, the temperature was continuously monitored in an auxiliary vessel in the temperature-controlled water bath.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used. Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution.	Sediment from all levels was analyzed for bifenthrin on Days 0 and 10. Following removal of the overlying water, the sediment was centrifuged at ≥10,000 *g* for 30 minutes and thoroughly mixed prior to analysis using GC-MSD based on methodology validated at Smithers Viscient (see Reviewer's Comments section for further details).

11. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes (see Reviewer's Comments).
Control Criteria Was control mortality ≤30%? Were control *C. tentans* an average size of ≥0.6 g?	 Negative control – 9% Solvent control – 12% N/A
Percent Recovery of Chemical:	Results of quality control (QC) samples fortified at 3.00, 30.0 or 120 µg/kg and analyzed concurrently with test samples: Sediment: 103 to 115% of nominal (n=6)
Data Endpoints - Survival - Dry weight (determined by pooling all living organisms from a replicate and drying at 60 to 90°C to a constant weight) - Body length (amphipod only)	 - Survival - Body length
Raw data included?	Yes, sufficient

Effects Data

Toxicant Concentration, µg/kg		Survival % ± SD	Length per amphipod mm ± SD
Nominal	Mean-Measured		
Negative Control	<LOQ[a]	91 ± 10	3.62 ± 0.10
Solvent Control	<LOQ	88 ± 9	3.50 ± 0.20
7.5	7.0	84 ± 9	3.58 ± 0.13
15	15	88 ± 10	3.45 ± 0.12*
30	31	76 ± 13*	3.10 ± 0.18 [b]
60	58	8 ± 7*	2.44 ± 0.39 [b]
120	130	0 ± 0*	--- [b]

[a] LOQ = 0.56 to 0.60 µg/kg.
[b] Excluded from statistical analysis due to significant effect on survival at this level.
* Statistically-significant compared to the negative control ($p<0.05$) based on Dunnett's Multiple Comparison Test.

Biological:

After 10 days, survival averaged 91 and 88% for the negative and solvent controls, respectively, and 84, 88, 76, 8 and 0% for the mean-measured 7.0, 15, 31, 58 and 130 µg/kg sediment treatment levels, respectively. Differences were statistically-significant compared to the negative control at the ≥31 µg/kg levels ($p<0.05$; Dunnett's Multiple Comparison Test). Using mean-measured concentrations, the NOAEC and LOAEC for survival were 15 and 31 µg/kg, respectively, and the 10-day LC_{50} (with 95% C.I.) was 40 (37 to 43) µg/kg. Adjusted for the organic carbon (OC) content of the sediment (i.e., 3.1%), the NOAEC and LOAEC for 10-Day survival were 0.48 and 1.0 µg/g OC, respectively, and the LC_{50} (with 95% C.I.) was 1.3 (1.2 to 1.4) µg/g OC.

Length at Day 10 averaged 3.62, 3.50, 3.58, 3.45, 3.10 and 2.44 mm per amphipod at the negative control, solvent control, and mean-measured 7.0, 15, 31, and 58 µg/kg treatment levels, respectively (there were no surviving amphipods at the 130 µg/kg treatment level). The difference was statistically-significant compared to the negative control at the 15 µg/kg level (higher levels were excluded from analysis due to significant effects on survival). Using mean-measured concentrations, the NOAEC and LOAEC for growth were 7.0 and 15 µg/kg, respectively. The observed 10-day EC_{50} was >58 µg/kg. Adjusted for the organic carbon (OC) content of the sediment (i.e., 3.1%), the NOAEC and LOAEC for 10-Day growth were 0.23 and 0.48 µg/g OC, respectively, and the EC_{50} was >1.9 µg/g OC.

Analytical:

Dosing stock solutions and treated sediment from all levels (prior to allocation into the replicate vessels) were analyzed for bifenthrin. Recoveries in the stock solutions ranged from 107 to 114% of nominal concentrations. Analysis of the spiked sediment following dosing and prior to allocation into the replicate exposure vessels ranged from 95 to 110% of nominal concentrations.

Bifenthrin sediment concentrations were relatively constant during the 10-day study. For the nominal 7.5, 15, 30, 60 and 120 µg/kg levels, Day-0 measured concentrations were 7.3, 15, 32, 57 and 120 µg/kg, respectively, and Day-10 measured concentrations were 6.8, 16, 31, 59 and 130 µg/kg, respectively. Mean-measured sediment concentrations were 7.0, 15, 31, 58 and 130 µg/kg, representing 94 to 110% of nominal levels. Adjusted for the organic carbon (OC) content of the sediment (i.e., 3.1%), the mean-measured concentrations were 0.23, 0.48, 1.0, 1.9 and 4.2 µg/g OC.

B. Statistical Results

Statistical analyses were performed on amphipod survival and growth (length) using CETIS™ (version 1.8, 2011) statistical software. Percent survival data were arcsine square-root transformed prior to analysis.

An Equal Variance Two-Sample t-Test was used to compare the performance of the negative control and solvent control data. Regardless of the results of the comparisons (not reported), the treatment groups were compared to the negative control data to determine potential treatment-related effects.

Data for both endpoints were tested for normality using the Shapiro-Wilks' Test and for homogeneity of variance using Bartlett's Test. Survival and length data met both assumptions and were subsequently analyzed using Dunnett's Multiple Comparison Test. Survival data were analyzed before length data, and any levels demonstrating a significant effect were excluded from subsequent analysis.

The NOAEC and LOAEC values were assigned based upon significance. All statistical analyses were conducted at the 95% level of certainty except in the case of the qualification tests (i.e., Shapiro-Wilks' and Bartlett's Tests), in which a 99% level of certainty was applied.

The Trimmed Spearman-Karber method within CETIS™ was used to calculate the LC_{50} value with associated 95% confidence intervals (C.I.).

Endpoint	Methods	Mean-measured Sediment, µg/kg	OC-normalized Sediment, µg/g OC
10-Day survival	Dunnett's Multiple Comparison Test	NOAEC: 15 LOAEC: 31 LC_{50}: 40 95% C.I.: 37 to 43	NOAEC: 0.48 LOAEC: 1.0 LC_{50}: 1.3 95% C.I.: 1.2 to 1.4
10-Day length	Dunnett's Multiple Comparison Test	NOAEC: 7.0 LOAEC: 15 EC_{50}: >58 95% C.I.: N/A	NOAEC: 0.23 LOAEC: 0.48 EC_{50}: >1.9 95% C.I.: N/A

12. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer analyzed data for survival and length. It should be noted that the current 850.1735 test design within CETIS only captures dry weight growth data, while length was the growth endpoint measured in this study; the reviewer entered the length data into the column for dry weight analysis. The negative and solvent control groups were compared for both endpoints using a two-sided equal variance t-test and no significant differences were detected. The reviewer compared treated groups to the negative control only. Data were tested to verify homogeneity of variances using Levene's (survival) or Bartlett's (length) tests and normality using Shapiro-Wilk's test. Length data did not satisfy parametric assumptions, while survival data did. The reviewer determined the NOAEC/LOAEC values using the William's (survival) and Jonckheere-Terpstra (length) tests. The LC_{50} was determined using linear regression and the EC_{50} for length was determined using nonlinear regression. These analyses were conducted using CETIS v. 1.8.7.12 with backend settings implemented by EFED on 3/25/14. The reviewer based toxicity estimates on the mean-measured sediment concentrations and additionally corrected values to reflect OC-normalized sediment concentrations.

Endpoint	Methods	Mean-measured Sediment, µg/kg	OC-normalized Sediment, µg/g OC
10-Day survival	William's Test Linear regression	NOAEC: 15 LOAEC: 31 LC_{50}: 41.2 95% C.I.: 37.2 to 44.8 Slope: 9.19 (6.69 to 11.7)	NOAEC: 0.48 LOAEC: 1.0 LC_{50}: 1.33 95% C.I.: 1.2 to 1.4

Endpoint	Methods	Mean-measured Sediment, µg/kg	OC-normalized Sediment, µg/g OC
10-Day length	Jonckheere-Terpstra Test	NOAEC: 7.0 LOAEC: 15	NOAEC: 0.23 LOAEC: 0.48
	Nonlinear regression	EC_{50}: 94.9 95% C.I.: 75.2 to 120	EC_{50}: 3.06 95% C.I.: 2.43 to 3.87

13. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with the study author's conclusions. In addition to mean-measured sediment concentrations, results were reported (by the study author) in terms of OC-normalized sediment concentrations. The organic carbon content of the natural sediment was 3.1%. Results were provided in terms of mean-measured sediment (bulk and OC-normalized) in the Conclusions section of the DER.

Nominal Sediment (µg/kg)	Mean-measured Sediment (µg/kg)	OC-Normalized Sediment (µg/g OC)
7.5	7.0	0.23
15	15	0.48
30	31	1.0
60	58	1.9
120	130	4.2

It was reported that pore water was not analyzed during this study due to the difficulty in accurate measurements of pyrethroids in pore water using standard methods. Overlying water was not analyzed due to the pyrethroids' strong affinity to sediment (i.e., high K_{oc} values) and regular renewal of the overlying water. It was also reported that previous studies performed at the laboratory indicated that only negligible amounts of pyrethroids partition to overlying water (Laboratories Study Nos. 13656.6106, 13656.6107, 13656.6110, 13656.6111, and 13656.6112, Putt, 2005).

All exposure and QC sediment samples were analyzed for bifenthrin using gas chromatography with mass selective detection (GC/MSD) based on methodology validated at Smithers Viscient. The method validation was conducted prior to the initiation of definitive testing and established an average recovery of 92.4 ± 10.8% from formulated sediment. It was reported that conditions and procedures used throughout the analysis of exposure solutions and QC samples during this study were similar to those used in the method validation with the following exception: the internal standard was changed to the stable isotope, d_6-bifenthrin. A method extension was also performed to verify recovery of bifenthrin at concentrations up to 2000 µg/kg; the method extension established an average recovery of 101 ± 8.25% from formulated sediment.

In addition to total hardness and specific conductivity, total alkalinity and ammonia were determined in the overlying water of each level on Days 0 and 10. Total alkalinity ranged from 14 to 18 mg/L as $CaCO_3$ during the study, and ammonia (as N) ranged from 0.67 to 1.2 mg/L on Day 0 and from 0.27 to 0.58 mg/L on Day 10.

The pH, ammonia (as N), dissolved organic carbon (DOC), and total organic carbon (TOC) were measured in a pore water sample obtained from each test level on Days 0 and 10. Throughout the study, the pH ranged from 5.7 to 5.9 and the DOC ranged from 9.0 to 14 mg/L. The TOC ranged from 190 to 290 mg/L on Day 0 and increased slightly to 270 to 350 mg/L on Day 10. Ammonia levels ranged from 5.6 to 6.3 mg/L on Day 0 and decreased slightly to 2.2 to 4.7 mg/L on Day 10.

This study was conducted in compliance with all pertinent U.S. EPA GLP regulations (40 CFR, Part 160) with the following exceptions: routine water, sediment, and food contaminant screening analyses. These analyses, however, were performed using certified laboratories and standard validated methods.

The experimental test dates were August 27 to September 18, 2013.

14. REFERENCES:

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